TO: Arizona Iceberg Lettuce Research Council
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Project Title: "Evaluation Arizona-Native Entomopathogenic Nematodes for Management of Lepidopterous Larvae in Iceberg Lettuce in Laboratory Experiments"

Introduction

Effective control of insect pests continues to be an overriding concern throughout all aspects of American life. The prevailing use of chemical pesticides has generated several problems including insecticide resistance, outbreaks of secondary pests, safety risks for humans and domestic animals, contamination of ground water, decrease in biodiversity among other environmental concerns (Lacey et al., 2001). These problems and sustainability programs based mainly on conventional insecticides have stimulated increase interest in the development and implementation of cost-effective, environmentally safe alternatives to chemical pesticides for insect pest control. Sustainable IPM in the 21st century will rely increasingly on alternative strategies for pest management that are environmentally friendly and reduce the amount of human contact to chemical pesticides. One of the most promising choices to help minimize usage of chemical pesticides is the implementation of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae).

Like other natural enemies, these insecticidal nematodes (hereafter refer to as EPN) can exert considerable control of target populations. Many species are employed as biological control agents of insect pests in row and glasshouse crops, orchards, ornamentals, range, turf and lawn, store products and forestry, as well as for control of pests and vectors of veterinary and medical importance around the world (Lacey et al., 2001). Interest in EPN has increased exponentially over the past years because of their many positive attributes. For example, they are effective control agents of many soil insects such as weevils, grubs, white grubs, mole crickets, and lepidopterans, etc. These insect pests are often controlled by chemical pesticides but now, EPN may be used for their suppression. Moreover, these insecticidal nematodes are widespread in nature, specific to insects, safe to non-target organisms including humans, other vertebrates, and plants, and do not pollute the environment. EPN can also be mass-produced in large fermentation tanks, can be stored for long periods and applied by conventional methods using standard spray equipment, making them a desirable commercial alternative (Georgis, 1990).

Several Arizonan EPN species (Heterorhabditidae and Steinernematidae) have recently been isolated (Stock et al., 2003) and are now available for testing against native insect pests. Several EPN species and isolates are known to be particularly susceptible to lepidopteran insects including beet armyworms, cabbage loopers, tobacco budworms and corn earworms (Raulston et al., 1992; Gaugler, 1999; Baur et al., 1997). Many studies have demonstrated the efficacy of EPN in controlling lepidopterous pests reducing significantly the rate of economic damage (Glaser et al., 1992; BenYakir et al., 1998)

Moreover, these indigenous isolates may provide a more suitable alternative for inundative release against a variety of native and/or introduced pests because of their adaptations to local climate conditions and population regulators.

Finally, control of lepidopteran pests in lettuce by EPN werenefit Arizona's vegetable crop industry by minimizing current usage of chemical pesticides, therefore providing a more environmentally friendly alternative for the management of this crop.

Methods

We tested 3 native EPN isolates: *Steinernema* sp. (ML8 isolate), *Steinernema oregonense* (BLP isolate) and *Heterorhabditis* sp (Chiricahua 35 isolate) against four commonly encountered lepidopteran pests in lettuce: cabbage looper (*Trichoplusia ni*), fall armyworm (*Spodoptera exigua*), corn earworm (*Heliothis zea*) and the black cutworm (*Agrotis ipsilon*).

For each of nematode/lepidopteran combinations we evaluated dose-response, exposure time, nematode penetration efficiency, nematode progeny production.

Dose-response essays: Two nematode inocula: a low (50IJs/host) and a high (500 IJs/insect) nematode density were considered. The one-on-one essay described by Kaya and Stock 91997) was considered for this essay. Tissue culture plates with 12 wells were filled wit 0.5 g of pasteurized air-dried sandy loan soil and placed in incubators set at 25 C. There were two tissue culture plates per treatment (dosage) plus a third one as the control. Bioassays were conducted inside plastic bags with wet paper towel to reduce moisture loss. After 1h acclimation, a 100 µl of nematode inoculum containing the tested nematode concentration suspension was added to each well in two of the plates and no nematodes in the third tray to serve d as a control for wax moth mortality. The plates were examined every 12 h for 7 days and the mortality of the wax moth larvae were recorded. To determine the time of death, each larva was probed, and if the larva did not respond to the probe, it was considered dead

Nematode penetration efficiency: Penetration efficiency of nematodes (no. of nematodes that successfully penetrate into the host) was evaluated using procedures described by Koppenhoffer and Kaya (1999). Two replicates and a control were considered. The enzymatic digestion method by Mauleon et al (1993) was used to dissect insect cadavers and record total no. of penetrating nematodes

Progeny production: Experimental set up was similar to the one described above for dose-response essays. traps (modified White traps) and consisted of a 35 x 10-mm Petri dish lid lined with one filter paper; with this lid floating on a sterilized deionized water in a 100 x 15-mm Petri dish (Kaya and Stock, 1997). The traps were kept in incubators at 15, 20, 25 and 30 ºC and were observed daily. The day of first emergence of nematodes (IJ) from the cadaver and the first day on which IJ migrate into the water were recorded. Emerged IJ were periodically harvested and kept in tissue culture flasks at 15 ºC. Emergence was stopped after 20 days. The number of IJ was estimated by counting four subsamples from the IJ suspension collected from each cadaver.

Exposure-time essay: The 12-well tissue plate arena was used for this experiment. A piece of filter paper was placed at the bottom of each well. Three plates were used/treatment. A nematode suspension of 8,000 nem/ml were prepared, and 50 ul of this suspension were pipeted to each the filter paper in each well. A single insect larva was added into each well and cells were sealed with their lid. After 3, 6 and 9 h insects were removed from each well, rinsed and transferred to clean 5cm petri dish added with filter paper. Dishes were stored at 25C for at least 40h. Insect mortality was recorded 48h after initial exposure. The entire essay was repeated 3 times. Et50 mortality (the time required to kill 50% of the exposed insects) were recorded

Analysis of data: Mortality data was corrected for control mortality following Abbot (1925), and arcsine transformed before analysis. Analysis of variance, probit analysis and means separation with Tukey's test (SigmaStat, Jandel Scientific) were used to analyze all comparisons.

Results:

Dose response and Insect Mortality: Both high and low nematode inocula were effective in causing insect larva mortality.Size of inocula did not seem to have a different effect on insect mortality. Steinernema oregonense (Bubbling Ponds isolate) was the most effective nematode strait tested, causing more than 80% mortality in cabbage looper (T. ni), Fall armyworm (S. exigua) and corn-earworm (H. zea) last instar larvae at the high dosis rate. At the lower rate this % mortality varied from 60-80% over a 5-day exposure period. Agrotis ipsilon (cutworms) were very resilent to the effect of any nematode strain at both doses tested. Heterorhabditis sp (CH-35 strain) was the best isolate in killing cutworm larvae in these experiments (25-30% mortality) (Figures 1 and 2).

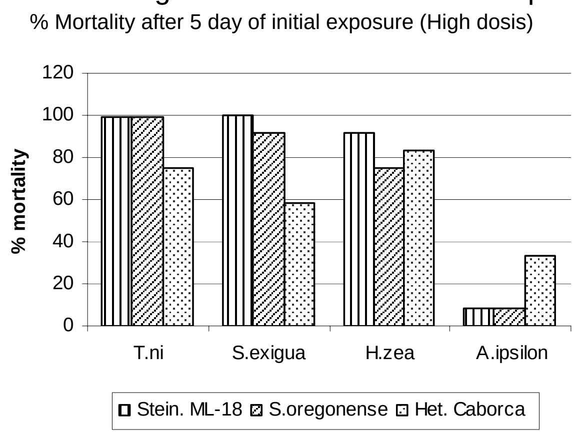

Figure 1.

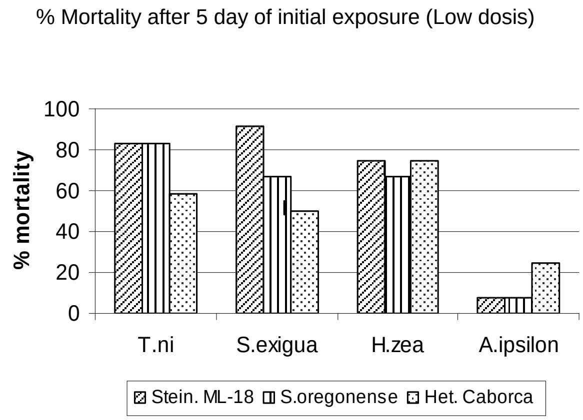

Figure 2.

Penetration efficiency: Penetration efficiency is evaluated as the total number of nematodes (infective juveniles or IJs) that manage to penetrate and kill an insect larva. In our experiments, the highest no. of established nematodes was attained by S. oregonense (Bubbling Ponds isolate) in corn-earworm larvae, with more than 30 IJs established for the highest. However, a good establishment of nematode IJs was also observed in the cabbage looper (>20 IJs/larva) and fall armyworm (>15 IJs/lava). Steinernematid isolates tested performed better than the Heterorhabditis isolate. As it was expected from our previous essays (i.e. dosis response), number of established IJs in cutworm larvae was the lowest (Figures 3 and 4).

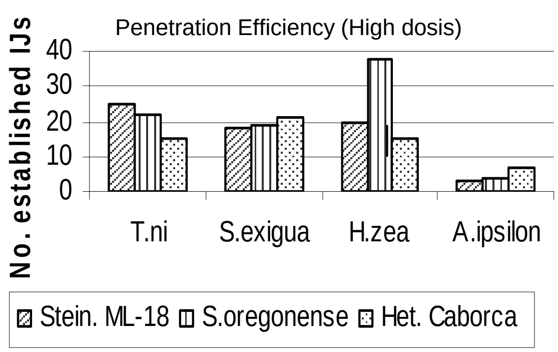

Figure 3.

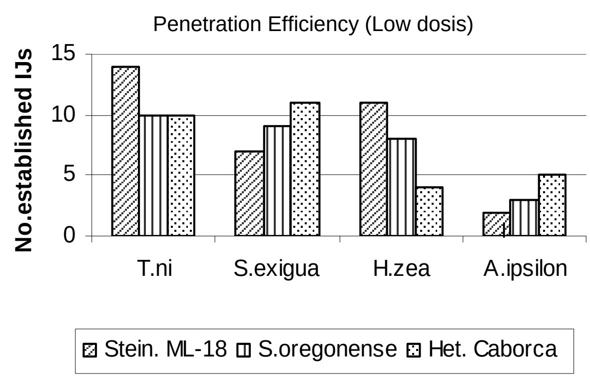

Figure 4.

Progeny Production: Progeny recovered from the high dosis essays showed that Steinernema sp. (ML-18 isolate) was the most successful in reproducing within fall armyworm and cabbage looper and corn earworm larvae. Steinernema oregonense (Bubbling Ponds isolate) also preformed well in producing juveniles in these three lepidopteran species, with and average of over 200,000 IJs/larvae. Production of progeny was very low in the black cutworm larvae. Steinernema sp. (ML-18 isolate) produced the maximum number of IJs (at high dosis), but this was less than 100,000.

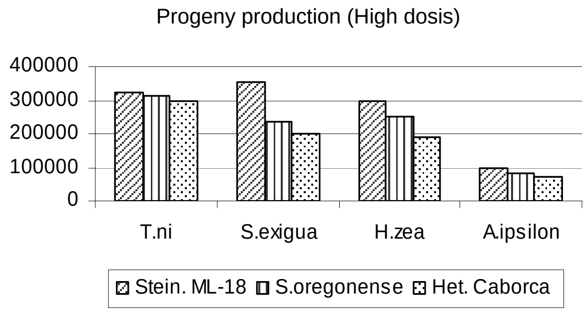

Figure 5

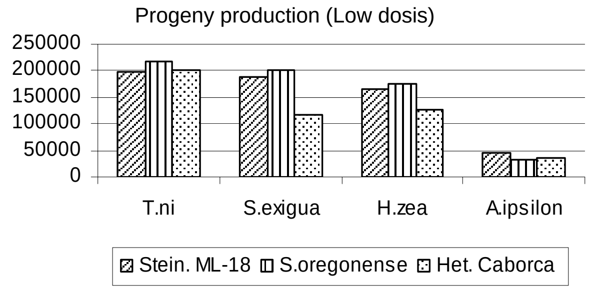

Figure 6.

Conclusions

From all Arizona-native nematode isolates tested Steinernematids performed better than the Heterorhabditis sp. This was reflected in the percent mortality, nematode establishment and progeny production. Three out of the four lepidopteran larvae (i.e. fall armyworms, cabbage loopers, and corn earworms) tested seem to be very susceptible to this nematode. Agrotis ipsilon (black cutworm) showed to be the most resilient insect to parasitism by any of the nematode isolated considered. However, this results are not surprising, other studies have shown similar results (Kaya et al., 1993, Caroli et al. 1996; Hussaini et al, 2002), indicating that perhaps this insect's immune system manages to evade nematode infection.

Overall, these essays show promise for consideration of Arizona-native entomopathogenic nematodes in the management of these lepidopteran pests in our state. It would be valuable if these studies could be expanded to greenhouse applications (for further evaluation of nematode dose response) and consideration of combined application of nematodes and currently used agrochemicals.